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Title of Invention: _____

Inventors (please provide full names): _____

_____

Earliest Priority Filing Date: _____

**For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

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Searcher: _____	NA Sequence (#) 2	STN _____
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PTO-1590 (1-2000)

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     (FILE 'HOME' ENTERED AT 07:20:01 ON 21 SEP 2001)

     FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT
     07:20:47 ON 21 SEP 2001
L1         6501 S (PYRUVATE OR PYRUVIC) (W) CARBOXYLASE
L2          136 S L1 AND CORYNE?
L3           48 S L2 NOT PY>1997
L4           28 DUP REM L3 (20 DUPLICATES REMOVED)

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Y/(N):y

L4 ANSWER 1 OF 28 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:184754 CAPLUS
DOCUMENT NUMBER: 128:292608
TITLE: Determination of the carbon flux in the central metabolism of **Corynebacterium** glutamicum by 13C-isotope analysis
AUTHOR(S): Marx, Achim
CORPORATE SOURCE: Inst. Biotechnologie, Forschungszentrum Juelich G.m.b.H., Juelich, D-52425, Germany
SOURCE: Ber. Forschungszent. Juelich (1997), Juel-3459, 1-111 pp.
CODEN: FJBEE5; ISSN: 0366-0885
DOCUMENT TYPE: Report
LANGUAGE: German
AB All C fluxes of the central metab. of C. glutamicum were quantified and the role and coordination of single metabolic pathways were studied under different metabolic situations. A method based on 13C-data was established to quantify all metabolite fluxes of the central metab. Strong sensitivities were indicated between metabolic fluxes and 13C data, thus allowing the detn. of metabolite flux. When the 13C-content of the position oxalacetate C-4 was varied by the factor 2 it could be shown if anaplerotic prodn. of C4-bodies was via the carboxylation of C3-bodies or via the glyoxalate cycle. A hyperbolic relationship was shown for the bi-directional turnover of transketolase and the 13C-content of the position pentose-5-phosphate C-1 and for the bi-directional metabolite flux between C3-bodies of glycolysis and C4-bodies of the tricarboxylate (TCA) cycle and 13C-enrichment of the position oxalacetate C-2. The NADPH balance showed that, depending on the conditions, more NADPH was produced than necessary for the synthesis of biomass and products. The NADPH excess was 16-67% in relation to the glucose uptake rate. Depending on the metabolic situation, the C4-body-decarboxylation was 10-132% and opposed to the carboxylation of C3-bodies for the anaplerotic supply of the TCA cycle. C4-body-decarboxylation and NADPH-excess as adaptations to high prodn. of Lys were minimal, with a yield coeff. of 0.32 molLysmolglucose-1. The contribution of malate enzyme to a total NADPH prodn. of 211% was small. The pentose phosphate pathway (PPP) and the TCA cycle produced 3/4 and 1/4, resp., of the total NADPH. Overexpression of glutamate dehydrogenase in a mutant of strain MH20-22B resulted in low TCA cycle flux and a high metabolite flux through the oxidative PPP. A high TCA cycle flux was detected during glutamate prodn. using strain LE4. The PPP flux was low in this strain. In a mutant of strain MH20-22B producing Lys and using NADH for synthesis of glutamate, TCA cycle flux was 79% and that of PPP was 26%. The low PPP was due to low NADPH consumption and high NADPH prodn. from isocitrate dehydrogenase of the TCA cycle. A strain ATCC 13032 isocitrate dehydrogenase mutant with a blocked TCA cycle showed a PPP flux of 62%. This mutant showed a glyoxalate cycle active in vivo when metabolizing glucose. This metabolite flux was 53%. A flux of 16% produced anaplerotically C4-bodies. At a flux of 37% the glyoxalate cycle released CO2 by C4-body decarboxylation and pyruvate dehydrogenase.

L4 ANSWER 2 OF 28 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:631539 CAPLUS
DOCUMENT NUMBER: 127:305184
TITLE: Physiological and NMR-spectroscopic investigations of in vivo activity of central metabolism pathways in wild and recombinant strains of **Corynebacterium** glutamicum
AUTHOR(S): Wendisch, Volker
CORPORATE SOURCE: Inst. Biotechnologie, Forschungszentrum Julich G.m.b.H., Juelich, D-52425, Germany
SOURCE: Ber. Forschungszent. Juelich (1997), Juel-3397, 1-111 pp.
CODEN: FJBEE5; ISSN: 0366-0885
DOCUMENT TYPE: Report
LANGUAGE: German
AB The C flux in the central metab. of C. glutamicum grown on glucose and/or acetate was detd. quant. and qual. The physiol. characterization of the growth of C. glutamicum revealed that this organism is able to metabolize acetate and glucose simultaneously. The C-uptake rates were quite similar with 900-1100 nmol C/mg protein. To analyze the C flux by 13C-labeling expts., a new NMR-spectroscopic method was developed, calibrated, and applied. This 1H-spin-echo-NMR method for the detn. of 13C-labeled non-protonated C-atoms, for example in carboxyl groups of amino acids, is 4-8-fold more precise than conventional NMR methods. Qual. C flux analyses revealed that beside the PEP-carboxylase C. glutamicum possesses another anaplerotic C3-carboxylating reaction, a **pyruvate carboxylase**. In addn., an alternative acetate activation to the acetate-kinase-phosphotransacetylase way was found in C. glutamicum which is suggested an acetyl-CoA-synthetase. The C fluxes in the central metab.

of C. glutamicum growing on glucose and/or acetate were quantified for the 1st time by 13C-labeling expts. and subsequent NMR-spectroscopic anal. of cellular amino acids in combination with the metabolite balance. The in vivo activities of the citrate synthase increased from 120 mU/mg protein on glucose to over 220 mU/mg protein on glucose plus acetate up to 410 mU/protein on acetate. The anaplerotic function was adopted by the PEP carboxylase and the **pyruvate carboxylase** at growth on glucose. At growth on acetate and surprisingly also at growth on glucose plus acetate, the glyoxylate cycle was active in vivo as the only anaplerotic sequence with 99 and 50 mU/mg protein, resp. The characterization of glyoxylate cycle-deficient C. glutamicum strains, which were produced by directed deletion of the genes for isocitrate lyase and the malate synthase, revealed that the glyoxylate cycle is essential for the optimal growth on glucose plus acetate. The glyoxylate cycle enzymes isocitrate lyase and malate synthase are regulated genetically by control of transcription of their genes aceA and aceB. High intracellular concns. of the metabolite acetyl-CoA correlated with high specific activities of the enzymes of the acetate metab.

L4 ANSWER 3 OF 28 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
ACCESSION NUMBER: 1997:301917 CAPLUS
DOCUMENT NUMBER: 127:2835
TITLE: **Pyruvate carboxylase** as an anaplerotic enzyme in **Corynebacterium** glutamicum
AUTHOR(S): Peters-Wendisch, Petra G.; Wendisch, Volker F.; Paul, Susanne; Eikmanns, Bernhard J.; Sahm, Hermann
CORPORATE SOURCE: Forschungszentrum Julich, Institut fur Biotechnologie, Julich, D-52425, Germany
SOURCE: Microbiology (Reading, U. K.) (1997), 143(4), 1095-1103
CODEN: MROBEO; ISSN: 1350-0872
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The recent discovery that phosphoenolpyruvate carboxylase (PEPCx) is dispensable for growth and lysine prodn. in **Corynebacterium** glutamicum implies that this organism possesses (an) alternative anaplerotic enzyme(s). In permeabilized cells of C. glutamicum, we detected **pyruvate carboxylase** (PCx) activity. This activity was effectively inhibited by low concns. of ADP, AMP and acetyl-CoA. PCx activity was highest [45 nmol min-1 (mg dry wt)-1] in cells grown on lactate or pyruvate, and was about 2-3 times lower when the cells were grown on glucose or acetate, suggesting that formation of PCx is regulated by the carbon source in the growth medium. In cells grown at low concns. of biotin (<5 .mu.g L-1), PCx activity was drastically reduced, indicating that the enzyme is a biotin protein. Growth expts. with the wild-type and a defined PEPCx-neg. mutant of C. glutamicum on glucose showed that the mutant has a significantly higher demand for biotin than the wild-type, whereas both strains have the same high biotin requirement for growth on lactate and the same low biotin requirement for growth on acetate. These results indicate that (i) PCx is an essential anaplerotic enzyme for growth on glucose in the absence of PEPCx, (ii) PCx is an essential anaplerotic enzyme for growth on lactate even in the presence of PEPCx, and (iii) PCx has no anaplerotic significance for growth on acetate as the carbon source. In support of these conclusions, screening for clones unable to grow on a minimal medium contg. lactate, but able to grow on a medium contg. glucose or acetate, led to the isolation of PCx-defective mutants of C. glutamicum.

L4 ANSWER 4 OF 28 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 97223734 MEDLINE
DOCUMENT NUMBER: 97223734 PubMed ID: 9056211
TITLE: Accurate determination of 13C enrichments in nonprotonated carbon atoms of isotopically enriched amino acids by 1H nuclear magnetic resonance.
AUTHOR: Wendisch V F; de Graaf A A; Sahm H
CORPORATE SOURCE: Institut fur Biotechnologie 1, Julich, Germany.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1997 Feb 15) 245 (2) 196-202.
Journal code: 4NK; 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970523
Last Updated on STN: 19990129
Entered Medline: 19970515
AB A method for the accurate determination of 13C enrichments in nonprotonated carbon atoms of organic compounds that makes use of unresolved 13C satellites of proton(s) bonded to the vicinal carbon atom

was developed. Using glutamate as a model molecule, this 1H nuclear magnetic resonance (NMR) inverse spin-echo difference spectroscopy method was calibrated for inversion efficiency and relaxation effects which were then shown to cause only a minor loss of the measured 13C satellite amplitude (2% for glutamate C-1 and 7% for glutamate C-5). The determination of 13C enrichments in nonprotonated glutamate carbon atoms by this method was shown to be more precise than 13C NMR. As a first application, a [5-13C]glucose labeling experiment with **Corynebacterium** glutamicum ASK1 was performed. The labeling patterns of glutamate and arginine extracted from cellular protein were determined using the newly developed method and standard 1H NMR with and without broadband 13C decoupling. Determination of the 13C enrichment in C-5 of glutamate and arginine, respectively, by the two methods showed good agreement. From the deduced labeling pattern of 2-oxoglutarate, an in vivo carbon flux distribution within the central metabolism of C. glutamicum ASK1 was calculated. Thus, the relative flux toward oxaloacetate via the tricarboxylic acid cycle enzyme malate dehydrogenase was determined as 45%, whereas that via anaplerotic C3 carboxylation was determined as 55%.

L4 ANSWER 5 OF 28 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:731636 CAPLUS
DOCUMENT NUMBER: 126:154946
TITLE: Anaplerotic reactions in **Corynebacterium** glutamicum. Studies of the significance of phosphoenolpyruvate (PEP)-carboxylase and **pyruvate-carboxylase** in the central metabolism and in amino acid production
AUTHOR(S): Peters-Wendisch, Petra
CORPORATE SOURCE: Inst. Biotechnol., Forschungszentrum Juelich G.m.b.H., Juelich, D-52425, Germany
SOURCE: Ber. Forschungszent. Juelich (1996), Juel-3259, 1-121 pp.
CODEN: FJBEE5; ISSN: 0366-0885
DOCUMENT TYPE: Report
LANGUAGE: German
AB Anaplerotic enzymes in C. glutamicum capable of C3-carboxylation were characterized and their role elucidated with respect to growth and prodn. of amino acids. It was shown by a defined phosphoenolpyruvate-carboxylase(PEPCx)- neg. mutant of C. glutamicum and the L-Lys-producing strain MH20-22B that PEPCx is not essential for growth and amino acid prodn. of C. glutamicum. H13CO3-labeling expts. revealed that the PEPCx-neg. mutant catalyzes the carboxylation of PEP or pyruvate. An IDP/GDP-depending PEP-carboxykinase was detected in C. glutamicum, catalyzing the carboxylation of PEP to oxaloacetate (activity of 80 mU/mg protein) and the decarboxylation of oxaloacetate to PEP (activity 1 mU/mg protein) in vitro. The low carboxylation activity and the inhibition by ATP and ADP in the anaplerotic direction indicate a gluconeogenetic function of the PEP-carboxykinase in vivo. The activity of **pyruvate-carboxylase** (PCx) in C. glutamicum was 15 mU/mg dry matter (DM) at growth on glucose and 50 mU/mg DM on lactate. The enzyme was inhibited by ADP, AMP, and acetyl CoA. The PCx is an anaplerotic enzyme alternative to PEPCx. It is essential at growth on lactate. PCx of C. glutamicum is a biotin enzyme with 120-130 kDa (denatured). A 17 kb HindIII-DNA-fragment was isolated carrying the gene for PCx. Compared with other PCx 60% of the resulting polypeptide with 451 amino acids were identical.

L4 ANSWER 6 OF 28 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3
ACCESSION NUMBER: 1996:88459 CAPLUS
DOCUMENT NUMBER: 124:115542
TITLE: Growth rate-dependent modulation of carbon flux through central metabolism and the kinetic consequences for glucose-limited chemostat cultures of **Corynebacterium** glutamicum
AUTHOR(S): Cocaign-Bousquet, Muriel; Guyonvarch, Armel; Lindley, Nicholas D.
CORPORATE SOURCE: Centre Bioingenierie Gilbert Durand, INSA, Toulouse, 31077, Fr.
SOURCE: Appl. Environ. Microbiol. (1996), 62(2), 429-36
CODEN: AEMIDF; ISSN: 0099-2240
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The physiol. behavior of **Corynebacterium** glutamicum in glucose-limited chemostat cultures was examd. from both growth kinetics and enzymic viewpoints. Metabolic fluxes within the central metab. were calcd. from growth kinetics and analyzed in relation to specific enzyme activities. At high growth rates, incomplete glucose removal was obsd., and this was attributed to rate-limiting capacity of the phosphotransferase system transporter and the probable contribution of a low-affinity permease uptake mechanism. The improved biomass yield obsd.

at high growth rates was related to a shift in the profile of anaplerotic carboxylation reactions, with **pyruvate carboxylase** replacing malic enzyme. Phosphoenolpyruvate carboxylase, an activity often assumed to be the major anaplerotic reaction during growth of C. glutamicum on glucose, was present at only low levels and is unlikely to contribute significantly to tricarboxylic acid cycle fuelling other than at low growth rates.

L4 ANSWER 7 OF 28 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 95154331 MEDLINE
DOCUMENT NUMBER: 95154331 PubMed ID: 7851427
TITLE: 13C-NMR studies of **Corynebacterium** melassecola metabolic pathways.
AUTHOR: Rollin C; Morgant V; Guyonvarch A; Guerquin-Kern J L
CORPORATE SOURCE: Centre ORSAN de Recherche en Biotechnologie, Les Ulis, France.
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1995 Jan 15) 227 (1-2) 488-93.
Journal code: EMZ; 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950322
Last Updated on STN: 19950322
Entered Medline: 19950313

AB **Coryneform** bacteria are widely used to produce amino acids, in particularly glutamic acid, by fermentation. To study the metabolic fate of glucose as the carbon source, we developed a method to analyze intracellular extracts by NMR and HPLC. The intracellular metabolites represent the metabolic state of the cells. Glutamic acid was the major metabolic intermediate found in the extracts and its 13C isotopic enrichment reflected that of pyruvic acid. Thus, it was possible to determine the respective contributions of the two major glucose catabolic pathways during the exponential growth phase; glycolysis (55%) and the pentose phosphate pathway (45%). Absolute glutamate 13C enrichments resulting from the incorporation of [1-13C]glucose were determined to quantify the contribution of several metabolic pathways such as anaplerotic pathways (61%; phosphoenolpyruvate carboxylase, **pyruvate carboxylase**, malic enzyme), a single turn (32%) or multiple turns of the Krebs cycle and the glyoxylate shunt, to oxaloacetate synthesis. A previously described model was adapted to C. melassecola for these calculations. The Krebs cycle was active, whereas the glyoxylate shunt was inactive in exponentially growing cells of C. melassecola with glucose as the sole carbon source. The contributions of anaplerotic enzymes and pyruvate dehydrogenase to replenishing the Krebs' cycle were determined to be 38% and 62%, respectively.

L4 ANSWER 8 OF 28 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 5
ACCESSION NUMBER: 1995:478620 CAPLUS
DOCUMENT NUMBER: 122:234995
TITLE: Pyruvate overflow and carbon flux within the central metabolic pathways of **Corynebacterium** glutamicum during growth on lactate
AUTHOR(S): Cocaign-Bousquet, Muriel; Lindley, Nicholas D.
CORPORATE SOURCE: Cent. Bioingen. Gilbert Durand, Inst. Natl. Sci. App., Toulouse, Fr.
SOURCE: Enzyme Microb. Technol. (1995), 17(3), 260-7
CODEN: EMTED2; ISSN: 0141-0229
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Growth of C. glutamicum was characterized kinetically for chemostat cultures using lactate as the sole C substrate, and the concn. profile of various enzymes of central metab. was established over a range of growth rates. Pyruvate overflow, together with incomplete lactate consumption, was obsd. under conditions for which the specific activity of pyruvate dehydrogenase no longer increased as a function of the growth rate. Biomass yields at such regimes were close to those obtained under exponential growth in batch cultures and were significantly higher than those assocd. with low growth rates. This shift in C conversion efficiency was correlated with increased malic enzyme and **pyruvate carboxylase** activities, suggesting the operation of a modified tricarboxylic acid cycle involving malate partitioning between malic enzyme (in conjunction with pyruvate carboxylation to replenish the C4 pool) and malate dehydrogenase. This metabolic deviation would provide the NADPH2 necessary to sustain the obsd. biomass yields in the absence of NADH:NADP transhydrogenase activity. An anal. of the energetic potential of such a metabolic network indicates that NADH oxidase plays an important role in modulating the respiratory chain efficiency so as to avoid excessive prodn. of ATP. C flux through the central metabolic pathways of

C. glutamicum is clearly a dynamic phenomenon whose regulatory complexity needs to be taken into account for future strain improvement strategies aimed at exploiting this organism's natural capacity to overproduce amino acids.

L4 ANSWER 9 OF 28 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 6
ACCESSION NUMBER: 1994:430822 CAPLUS
DOCUMENT NUMBER: 121:30822
TITLE: Effects of phosphoenol **pyruvate carboxylase** deficiency on metabolism and lysine production in **Corynebacterium** glutamicum
AUTHOR(S): Gubler, Marcel; Park, Sung Min; Jetten, Mike; Stephanopoulos, Gregory; Sinskey, Anthony J.
CORPORATE SOURCE: Dep. Biol., Massachusetts Inst. Technol., Cambridge, MA, 02139, USA
SOURCE: Appl. Microbiol. Biotechnol. (1994), 40(6), 857-63
CODEN: AMBIDG; ISSN: 0175-7598
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The phosphoenol **pyruvate carboxylase** (PEP carboxylase) gene (ppc) of lysine-producing **Corynebacterium** glutamicum and C. lactofermentum strains was inactivated by marker exchange mutagenesis. The mutants lacked completely PEP carboxylase activity, but grew in minimal medium contg. glucose as the sole carbon source. In addn., the ppc- strains produced equiv. titers of lysine in shake flasks and 10-L fermn. expts. as their parent strains. To address the question of how ppc- **Corynebacterium** strains generate oxaloacetate (OAA) for their own metab. as well as for high-level lysine prodn., the authors measured the activities of enzymes leading to OAA synthesis. Whereas **pyruvate carboxylase** activity was not detected in any of the strains, phosphoenol pyruvate carboxykinase (PEP carboxykinase) activity was found to be significantly higher in C. glutamicum ppc mutants compared to the parent strains. On the other hand, PEP carboxykinase activity in C. lactofermentum was essentially absent. As glyoxylate cycle enzymes are strongly repressed by glucose, they are not likely to compensate for the lack of PEP carboxylase activity. PEP carboxykinase, among several candidates, could play this role.

L4 ANSWER 10 OF 28 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 7
ACCESSION NUMBER: 1994:453650 CAPLUS
DOCUMENT NUMBER: 121:53650
TITLE: Regulation of phospho(enol)pyruvate- and oxaloacetate-converting enzymes in **Corynebacterium** glutamicum
AUTHOR(S): Jetten, Mike S. M.; Pitoc, George A.; Follettie, Maximillian T.; Sinskey, Anthony J.
CORPORATE SOURCE: Dep. Biol., Massachusetts Inst. Technol., Cambridge, MA, 02139, USA
SOURCE: Appl. Microbiol. Biotechnol. (1994), 41(1), 47-52
CODEN: AMBIDG; ISSN: 0175-7598
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The presence and properties of the enzymes involved in the synthesis and conversion of phospho(enol)pyruvate (PEP) and oxaloacetate (OAA), the precursors for aspartate-derived amino acids, were investigated in three different **Corynebacterium** strains. This study revealed the presence of both PEP carboxykinase (0.29 .mu.mol.min-1.mg-1 of protein [units (U).mg-1]) and PEP synthetase (0.13 U.mg-1) in C. glutamicum as well as pyruvate kinase (1.4 U.mg-1) and PEP carboxylase (0.16 U.mg-1). With the exception of PEP carboxykinase, these activities were also present in glucose-grown C. flavum and C. lactofermentum. **Pyruvate carboxylase** activity was not detected in all three species cultivated on glucose or lactate. At least five enzyme activities that utilize OAA as a substrate were detected in crude exts. of C. glutamicum: citrate synthase (2 U.mg-1), malate dehydrogenase (2.5 U.mg-1), glutamate:OAA transaminase (1 U.mg-1), OAA-decarboxylating activity (0.89 U.mg-1) and the previously mentioned PEP carboxykinase (0.29 U.mg-1). The partially purified OAA-decarboxylase activity of C. glutamicum was completely dependent on the presence of inosine diphosphate and Mn2+, had a Michaelis const. (Km) of 2.0 mM for OAA and was inhibited by ADP and CoA (CoA). Examn. of the kinetic properties showed that adenine nucleotides and CoA derivs. have reciprocal but reinforcing effects on the enzymes catalyzing the interconversion of pyruvate, PEP and OAA in C. glutamicum. A model for the regulation of the carbon flow based on these findings is presented.

L4 ANSWER 11 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1991:317358 BIOSIS
DOCUMENT NUMBER: BA92:27873
TITLE: ROLE OF BIOTIN IN GLUTAMATE BIOSYNTHESIS WITH A MIXED

SUBSTRATE OF GLUCOSE AND ACETATE.
AUTHOR(S): KANEGAE Y; NAKATSUI I; SUGIYAMA Y; KANZAKI T
CORPORATE SOURCE: VITAMIN AND FOOD RES. LAB., TAKEDA CHEMICAL INDUSTRIES, 1-5 TAKASAGO-CHO, TAKASGO-SHI,, HYOGO 676, JPN.
SOURCE: NIPPON NOGEIKAGAKU KAISHI, (1991) 65 (4), 737-746. CODEN: NNKKAA. ISSN: 0002-1407.
FILE SEGMENT: BA; OLD
LANGUAGE: Japanese

AB Brevibacterium thiogenitalis D-248, an effective glutamate producer which requires oleate but not biotin for its growth, was grown on a mixed carbon source with a mole ratio of glucose-to-acetate of 1:2. Glutamate accumulated drastically as biotin concentration in the medium was raised. The minimum concentration of biotin necessary for maximum production of glutamate was around 150 .mu.g/liter, at which concentration a glutamic acid yield as high as 70% (by weight) was attained. At concentrations less than 20 .mu.g/liter, D-248 failed to utilize acetate but consumed glucose to give pyruvate even in the presence of thiamine. Consequently no significant accumulation of glutamate was observed. Cell free extracts of biotin-sufficient cells had both phosphoenolpyruvate carboxylase (PEPC) and **pyruvate carboxylase** (PC) activities. The activity of PC was totally dependent on the biotin concentration in the medium, while that of PEPC remained roughly constant irrespective of the biotin concentration. These results and additional data provided evidence for the direct participation of biotin in glutamate biosynthesis through formation of an active holoenzyme of PC, which in turn may replenish the TCA cycle with oxaloacetate in situ.

L4 ANSWER 12 OF 28 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 8
ACCESSION NUMBER: 1990:586078 CAPLUS
DOCUMENT NUMBER: 113:186078
TITLE: Cloning of phosphoenol **pyruvate carboxylase** gene of **Corynebacterium** glutamicum for use in the fermentatin of amino acids
INVENTOR(S): Bachmann, Bernd; Thierbach, Georg; Kalinowski, Joern; Puehler, Alfred; O'Reagan, Mike; Viret, Jean Francois; Lepage, Pierre; Lemoine, Yves
PATENT ASSIGNEE(S): Degussa A.-G., Fed. Rep. Ger.
SOURCE: Eur. Pat. Appl., 27 pp. CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 358940	A1	19900321	EP 1989-114632	19890808
EP 358940	B1	19950913		
R: BE, DE, ES, FR, GB, IT, NL, SE				
GB 2223754	A1	19900418	GB 1988-21319	19880912
GB 2223754	B2	19920722		
JP 02291276	A2	19901203	JP 1989-234830	19890912
PRIORITY APPLN. INFO.:			GB 1988-21319	19880912

AB The ppc gene of **Corynebacterium** glutamicum encoding phosphoenolpyruvate carboxylase (I) is cloned in Escherichia coli and expressed in both of these organisms. High levels of expression of this gene result in high levels of oxaloacetate in the organism. As a result, the synthesis of aspartate and glutamate-derived amino acids is stimulated. The gene was cloned from a C. glutamiucum ATCC13032 bank (Sau3AI partial digest in pUC18) by complementation of the corresponding defect in E. coli. I-activity was readily detectable in E. coli transformants with the C. glutamicum enzyme distinguishable by not being stimulated by exogenous acetyl CoA. High-level expression of the gene in C. glutamicum (at up to 4 times the normal level) resulted in raising of levels of excretion of lysine, threonine and isoleucine by ~15%.

L4 ANSWER 13 OF 28 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1991:407004 CAPLUS
DOCUMENT NUMBER: 115:7004
TITLE: Manufacture of aromatic amino acids with **coryneform** bacteria
INVENTOR(S): Katsumata, Ryoichi; Kino, Kunikata
PATENT ASSIGNEE(S): Kyowa Hakko Kogyo Co., Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 7 pp. CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

JP 02303495	A2	19901217	JP 1989-123463	19890517

AB Tryptophan (I), tyrosine (II), or phenylalanine (III) is manufd. by cultivation of glutamic acid-producing **coryneform** bacteria that is lack of or low in phosphoenol **pyruvate carboxylase** and pyruvate kinase. **Corynebacterium** glutamicum K81 (FERM BP-2409, prepd. by treatment of C. glutamicum BPS13 with N-methyl-N'-nitro-N-nitrosoguanidine) was shake-cultured in a medium contg. glucose, polypeptone, yeast ext., urea, II, III, and NaCl at 30.degree. for 24 h and further cultured in a medium contg. glucose, biotin, corn steep liquor, and salts for 72 h to produce 8.5 mg I/L, vs. 7.8 mg/L, for the parent strain.

L4 ANSWER 14 OF 28 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 9
ACCESSION NUMBER: 1990:196674 CAPLUS
DOCUMENT NUMBER: 112:196674
TITLE: Enhanced manufacture of tryptophan, tyrosine, and phenylalanine with **coryneform** glutamic acid-producing bacterium mutant
INVENTOR(S): Katsumata, Ryoichi; Kino, Kuniki
PATENT ASSIGNEE(S): Kyowa Hakko Kogyo Co., Ltd., Japan
SOURCE: Eur. Pat. Appl., 8 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 331145	A2	19890906	EP 1989-103578	19890301
EP 331145	A3	19900829		
EP 331145	B1	19941109		
R: DE, FR, GB				
JP 01317395	A2	19891222	JP 1988-241688	19880927
JP 2578488	B2	19970205		
US 5484716	A	19960116	US 1993-130995	19931004
US 5595906	A	19970121	US 1995-429472	19950427

PRIORITY APPLN. INFO.:

JP 1988-51358	19880304
JP 1988-241688	19880927
US 1989-317589	19890301
US 1990-625699	19901212
US 1993-130995	19931004

AB A method for manufg. the title amino acids with **coryneform** glutamic acid-producing bacterium mutant that has reduced phosphoenol-**pyruvate carboxylase** (I) activity or lacks I activity is described. **Corynebacterium** glutamicum, a tryptophan-producing **coryneform** bacteria, was mutated by std. methods to obtain a mutant (BPS-13) that only contained 25% I activity of the parent strain. C. glutanicum BPS-13 was shake-cultured in a medium contg. glucose, corn steep liquor, and salts for 72 h to produce tryptophan 7.8 mg/mL culture medium. Grown in the same medium for 72 h, the parent strain produced tryptophan 6.0 mg/mL culture medium.

L4 ANSWER 15 OF 28 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1986-341462 [52] WPIDS
DOC. NO. CPI: C1986-148053
TITLE: New DNA fragment and recombinant molecules - contg. gene for phospho enol **pyruvate carboxylase** from **corynebacterium** useful for increasing yield of amino acid or nucleic acid.
DERWENT CLASS: B04 D16
INVENTOR(S): FUJII, M; ISSHIKI, S; TAKEDA, Y
PATENT ASSIGNEE(S): (ASAH) ASAHI KASEI KOGYO KK
COUNTRY COUNT: 2
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
FR 2581653	A	19861114	(198652)*		111
JP 62055089	A	19870310	(198715)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
FR 2581653	A	FR 1986-606712	19860509
JP 62055089	A	JP 1986-104768	19860509

PRIORITY APPLN. INFO: JP 1985-99200 19850510; JP 1986-104768

19860509
AN 1986-341462 [52] WPIDS
AB FR 2581653 A UPAB: 19930922
New DNA fragment (I) is derived from a bacterium of the genus **Corynebacterium**, which can produce glutamic acid, and contains a gene coding for phosphoenolpyruvate carboxylase (PEPC). Esp. (I) is derived from C. melassecola 801 (FERM BP-558).
Also new are (A) recombinant DNA consisting of (I) connected directly or indirectly to a second fragment (II) contg. a gene for replication in bacterial cells and (B) bacteria contg. recombinant DNA.
USE/ADVANTAGE - Bacteria contg. (II) have enhanced PEPC activity and can produce amino acids, nucleic acids, etc. in high yield.

L4 ANSWER 16 OF 28 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1986-114324 [18] WPIDS
DOC. NO. CPI: C1986-048717
TITLE: Fermentative prodn. of amino acids - using **Coryneform** bacteria transformed with two recombinant Plasmid(s) contg. enzyme genes.
DERWENT CLASS: B05 D16 E19
INVENTOR(S): MATSUI, K; MIWA, K; OOSUMI, C; SANO, K
PATENT ASSIGNEE(S): (AJIN) AJINOMOTO KK
COUNTRY COUNT: 6
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 179338	A	19860430	(198618)*	EN	51
R: DE FR GB					
JP 61088889	A	19860507	(198625)		
US 4980285	A	19901225	(199103)		
EP 179338	B1	19930811	(199332)	EN	18
R: DE FR GB					
DE 3587519	G	19930916	(199338)		
JP 07046994	B2	19950524	(199525)		11

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 179338	A	EP 1985-112621	19851004
JP 61088889	A	JP 1984-208677	19841004
US 4980285	A	US 1989-412562	19890925
EP 179338	B1	EP 1985-112621	19851004
DE 3587519	G	DE 1985-3587519	19851004
		EP 1985-112621	19851004
JP 07046994	B2	JP 1984-208677	19841004

FILING DETAILS:

PATENT NO	KIND		PATENT NO
DE 3587519	G	Based on	EP 179338
JP 07046994	B2	Based on	JP 61088889

PRIORITY APPLN. INFO: JP 1984-208677 19841004
AN 1986-114324 [18] WPIDS
AB EP 179338 A UPAB: 19930922
Prodn. of an L-amino acid (I) is effected by inserting a gene coding for an enzyme E1 into a plasmid vector P1, inserting a gene coding for an enzyme E2 into a plasmid vector P2, introducing the recombinant plasmids into a strain of **coryneform** bacteria, and culturing the transformant. E1 and E2 are different enzymes, each highly rate-determining in the biosynthesis of (I). P1 and P2 have compatible replicating orgins different from each other.
For threonine prodn., E1 is phosphoenol **pyruvate carboxylase** (PEPC) or homoserine kinase and E2 is homoserine dehydrogenase (HD). For tryptophan prodn., E1 is a combination of shikimate kinase and 3-dehydroquinate synthetase and E2 is tryptophan synthetase. Suitable plasmid vectors are pAM330, pAM1519, pAJ655, pAJ611 and pAJ1844. Suitable hosts include Brevibacterium and Corynbacterium strains producing L-glutamic acid.
0/0
ABEQ US 4980285 A UPAB: 19930922
Prodn. of L-aminoacids comprises inserting two or more genes that encode the formation of at least two enzymes required for the biosynthesis of an L-aminoacids into plasmid vectors; transformation of suitable host cells with these vectors; selective propagation of the transformed species; and isolation and purificn. of the L-aminoacid produced. E.g., plasmids pAJ210 and pAJ201K transform Brevibacterium species to produce L-threonine; pAJ1220 and pAJ319 transform Brevibacterium species to produce

L-tryptophane; and similarly, **Corynebacterium** species are transformed to express vital L-aminoacids.
USE - The process provides a convenient means of biosynthesis of L-aminoacids.

ABEQ EP 179338 B UPAB: 19931118
A method for producing an L-amino acid, which comprises: inserting at least one gene which codes for an enzyme which is utilized on the route of biosynthesis of an L-amino acid product into one of at least two plasma vectors which have compatible origins of replication different from each other; inserting at least one gene which codes for an enzyme different from said first group of enzyme(s) on the route of biosynthesis of said L-amino acid into another one of said plasmid vectors; introducing the thus obtained recombinant plasmids into a strain of **coryneform** bacteria of the aerobic, gram-positive, non-acid fast rod type; and culturing the thus transformed strain which is capable of producing the said L-amino acid, said two enzymes being highly rate determining enzymes for the biosynthesis of said L-amino acid.
Dwg.0/0

L4 ANSWER 17 OF 28 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1985-136176 [23] WPIDS
DOC. NO. CPI: C1985-059258
TITLE: Recombinant DNA contg. phospho enol **pyruvate carboxylase** gene - useful in **corynebacterium** and brevibacterium bacteria for improved aminoacid prodn..
DERWENT CLASS: B04 D16
INVENTOR(S): ITO, A T R; MIWA, K; NAKAMORI, S; SANO, K; ITO, K
PATENT ASSIGNEE(S): (AJIN) AJINOMOTO KK
COUNTRY COUNT: 7
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 143195	A	19850605	(198523)*	EN	48
R: DE FR GB IT NL					
JP 60087788	A	19850517	(198526)		
US 4757009	A	19880712	(198830)		
EP 143195	B1	19920527	(199222)	EN	20
R: DE FR GB IT NL					
DE 3485750	G	19920702	(199228)		
JP 07083714	B2	19950913	(199541)		11

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 143195	A	EP 1984-110141	19840824
JP 60087788	A	JP 1983-157512	19830829
US 4757009	A	US 1984-645107	19840828
EP 143195	B1	EP 1984-110141	19840824
DE 3485750	G	DE 1984-3485750	19840824
		EP 1984-110141	19840824
JP 07083714	B2	JP 1983-157512	19830829

FILING DETAILS:

PATENT NO	KIND		PATENT NO
DE 3485750	G	Based on	EP 143195
JP 07083714	B2	Based on	JP 60087788

PRIORITY APPLN. INFO: JP 1983-157512 19830829
AN 1985-136176 [23] WPIDS
AB EP 143195 A UPAB: 19930925
Recombinant DNA molecule (I) comprising a plasmid vector and a gene coding for phosphoenol **pyruvate carboxylase** (PEPC) operatively inserted in it is new. (I) is capable of propagation in, and the gene is capable of expression in, a **Corynebacterium** or Brevibacterium strain.
Corynebacterium or Brevibacterium strain carrying (I) and expressing both the gene it contains and a chromosomal gene coding for an amino acid is new.
USE/ADVANTAGE - Increased prodn. of amino acids such as lysine, threonine, isoleucine etc. from aspartic acid is achieved, as the PEPC improves formation of metabolic aspartic acid on cultivation of the bacteria.
0/8
ABEQ DE 3485750 G UPAB: 19930925
Recombinant DNA molecule (I) comprising a plasmid vector and a gene coding for phosphoenol **pyruvate carboxylase** (PEPC)

operatively inserted in it is new. (I) is capable of propagation in, and the gene is capable of expression in, a **Corynebacterium** or Brevibacterium strain.

Corynebacterium or Brevibacterium strain carrying (I) and expressing both the gene it contains and a chromosomal gene coding for an amino acid is new.

USE/ADVANTAGE - Increased prodn. of amino acids such as lysine, threonine, isoleucine etc. from aspartic acid is achieved, as the PEPC improves formation of metabolic aspartic acid on cultivation of the bacteria.

ABEQ EP 143195 B UPAB: 19930925

A recombinant DNA molecule formed of a plasmid vector and a gene coding for phosphoenol **pyruvate carboxylase** (PEPC gene) operationally inserted therein, said gene being obtainable from bacteria resistant to aspartic acid antagonists, wherein said recombinant DNA molecule is capable of propagating and said gene is capable of being expressed in a bacterium of the genus **Corynebacterium** and Brevibacterium, with the exception of (1) the recombinant DNA obtained by inserting the PEPC gene of E. coli into pBR322 and (2) the recombinant DNA, obtained from strain ATCC 39033, which is the ligation product of the recombinant plasmid (1) with the plasmid pCG11 (obtained from strain ATCC 39022).

ABEQ US 4757009 A UPAB: 19930925

Chromosomal gene which includes a gene coding for phosphoenol **pyruvate carboxylase** (PEPC) has been isolated from **Corynebacterium** and/or Brevibacterium strains. Recombinant DNA comprises a plasmide vector with gene coding for PEPC, which can be expressed in **Corynebacterium** and/or Brevibacterium species.

USE - The prods. (partic. PEPC) are utilised in the biochemical prodn. of aminoacids in much improved yields.

L4 ANSWER 18 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1985:406298 BIOSIS
DOCUMENT NUMBER: BA80:76290
TITLE: PRODUCTION OF L LYSINE FLUOROPYRUVATE-SENSITIVE MUTANTS OF BREVIBACTERIUM-LACTOFERMENTUM.
AUTHOR(S): TOSAKA O; YOSHIHARA Y; IKEDA H; TAKINAMI K
CORPORATE SOURCE: CENT. RES. LAB. AJINOMOTO CO., KAWASAKI 201, JAPAN.
SOURCE: AGRIC BIOL CHEM, (1985) 49 (5), 1305-1312.
CODEN: ABCHA6. ISSN: 0002-1369.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Better producers of L-lysine were obtained by derivation of fluoropyruvate(FP)-sensitive mutants from B. lactofermentum AJ3990. The coexistence of FP and excess biotin synergistically stimulated L-lysine formation by washed cells. FP inhibited 50% of growth and pyruvate dehydrogenase (PDH) activity of AJ3990 at 0.04 and 1 mM, respectively. The synergistic effect of FP and excess biotin seems to be due to the optimization of the PDH/**pyruvate carboxylase** activity ratio in L-lysine biosynthesis. This was confirmed by the derivation of FP-sensitive mutants which have the optimal level of PDH activity for L-lysine production. The best producer, AJ11204, had .apprx. 27% PDH activity, as compared with the parental strain and accumulated 70 g of L-lysine/l with a conversion yield of 50% from glucose in the presence of excess biotin.

L4 ANSWER 19 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1984:358334 BIOSIS
DOCUMENT NUMBER: BA78:94814
TITLE: PRODUCTION OF ASPARTIC-ACID AND ENZYMATIC ALTERATION IN PYRUVATE KINASE MUTANTS OF BREVIBACTERIUM-FLAVUM.
AUTHOR(S): MORI M; SHIIO I
CORPORATE SOURCE: CENTRAL RES. LAB., AJINOMOTO CO. INC., KAWASAKI-KU, KAWASAKI, KANAGAWA 210, JPN.
SOURCE: AGRIC BIOL CHEM, (1984) 48 (5), 1189-1198.
CODEN: ABCHA6. ISSN: 0002-1369.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB A pyruvate kinase-lacking mutant of B. flavum produced 22.6 g/l of L-aspartic acid, with glutamic acid as a by-product, when cultured for 48 h in a medium containing 100 g/l of glucose. The production clearly depended on the amount of biotin added. This strain, 70, was derived by several steps of mutation from wild strain 2247 producing glutamate, successively via a citrate synthase-defective glutamate auxotroph, strain 214, a prototrophic revertant, strain 15-8, producing 10 g/l of L-aspartic acid, and an S-(2-aminoethyl)-L-cysteine-resistant mutant, strain 1-231, having low pyruvate kinase and homoserine dehydrogenase and producing lysine. Strain 70, a methionine-insensitive revertant from strain 1-231, had a normal level of homoserine dehydrogenase but not pyruvate kinase. Its citrate synthase activity was .apprx. 1/2 that of the wild strain at saturated concentrations of the substrates, with Km for oxalacetate and

acetyl-CoA 110 and 6 times as high as those of the wild-type enzyme, respectively. The mutational step for these alterations in citrate synthase was strain 15-8. Phosphoenolpyruvate carboxylase of strain 70 showed 1.5-fold higher activity in the crude extract at saturated concentrations of phosphoenolpyruvate, a lower Km (1.5 mM) for phosphoenolpyruvate, less sensitivity to feedback inhibition by aspartate, and higher sensitivities to the activators acetyl-CoA and fructose-1,6-bisphosphate than those of the wild strain. The concentration of aspartate giving 50% inhibition were 6.2- and 4.5-fold higher in the absence and presence of acetyl-CoA, respectively.

L4 ANSWER 20 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1982:269547 BIOSIS
DOCUMENT NUMBER: BA74:42027
TITLE: CARBON-13 NMR STUDIES OF THE BIOSYNTHESIS BY MICROBACTERIUM-AMMONIAPHILUM OF L GLUTAMATE SELECTIVELY ENRICHED WITH CARBON-13.
AUTHOR(S): WALKER T E; HAN C H; KOLLMAN V H; LONDON R E; MATWIYOFF N A
CORPORATE SOURCE: LOS ALAMOS NATIONAL LAB., UNIV. CALIFORNIA, LOS ALAMOS, NEW MEXICO 87545.
SOURCE: J BIOL CHEM, (1982) 257 (3), 1189-1195.
CODEN: JBCHA3. ISSN: 0021-9258.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB 13C-NMR of isotopically enriched metabolites was used to study the metabolism of M. ammoniaphilum, a bacterium which excretes large quantities of L-glutamic acid into the medium. Biosynthesis from 90% [1-13C]glucose results in relatively high specificity of the label, with [2,4-13C2]glutamate as the major product. The predominant biosynthetic pathway for synthesis of glutamate from glucose was determined to be the Embden-Meyerhof glycolytic pathway followed by phospho-enolpyruvate carboxylase and the 1st third of the Krebs cycle. Different metabolic pathways are associated with different correlations in the enrichment of the Ca, reflected in the spectrum as different 13C-13C scalar multiplet intensities. Intensity and 13C-13C multiplet analysis allows quantitation of the pathways involved. Although blockage of the Krebs cycle at the .alpha.-ketoglutarate dehydrogenase step is the basis for the accumulation of glutamate, significant Krebs cycle activity was found in glucose grown cells, and extensive Krebs cycle activity in cells metabolizing [1-13C]acetate. In addition to the observation of the expected metabolites, the disaccharide .alpha.,.alpha.-trehalose and .alpha.,.beta.-glucosylamine were identified from the 13C-NMR-spectra.

L4 ANSWER 21 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1983:178937 BIOSIS
DOCUMENT NUMBER: BA75:28937
TITLE: PHOSPHOENOL **PYRUVATE CARBOXYLASE** EC-4.1.1.31 AND ITS OPERATION IN BREVIBACTERIUM-FLAVUM PRODUCING LYSINE.
AUTHOR(S): RUKLISH M P; MARAUSKA D F; LABANE L YA
CORPORATE SOURCE: A. KIRCHENSTEIN INST. MICROBIOL., ACAD. SCI. LATV. SSR, RIGA, USSR.
SOURCE: MIKROBIOLOGIYA, (1982) 51 (1), 17-20.
CODEN: MIKBA5. ISSN: 0026-3656.
FILE SEGMENT: BA; OLD
LANGUAGE: Russian
AB Phosphoenol **pyruvate carboxylase** (PEP-c) (EC 4.1.1.31) was shown to be the only enzyme catalyzing anapleurotic synthesis of oxalacetic acid in B. flavum synthesizing lysine. Acetyl-CoA was required for the operation of PEP-c in the strains. Changes in the activity of PEP-c did not entirely correlate with those of the citric acid cycle enzymes. Hence, PEP-c is involved not only in the citric acid cycle but also in other functions of the cell. A correlation was found between changes in the activity of PEP-c, the enzymes of the citric acid cycle and lysine production in B. flavum.

L4 ANSWER 22 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1983:35987 BIOSIS
DOCUMENT NUMBER: BR24:35987
TITLE: CHARACTERISTICS OF THE FUNCTIONING OF PHOSPHOENOL **PYRUVATE CARBOXYLASE** EC-4.1.1.31 IN BACTERIA BREVIBACTERIUM-FLAVUM PRODUCING LYSINE.
AUTHOR(S): RUKLISH M R; MARAUSKA D F; LABANE L YA
CORPORATE SOURCE: A. KIRCHENSTEIN INSTITUTE MICROBIOL., ACADEMY SCIENCES LATVIAN SSR.
SOURCE: Microbiology (Engl. Transl.), (1982) 51 (1), 12-15.
CODEN: MIBLAO. ISSN: 0026-2617.
FILE SEGMENT: BR; OLD
LANGUAGE: English

L4 ANSWER 23 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1980:223550 BIOSIS
DOCUMENT NUMBER: BA70:16046
TITLE: YIELD REGULATION OF LYSINE BIOSYNTHESIS IN BREVIBACTERIUM-FLAVUM.
AUTHOR(S): SHVINKA J; VIESTURS U; RUKLISHA M
CORPORATE SOURCE: AUGUST KIRCHENSTEIN INST. MICROBIOL., ACAD. SCI. LATV. SSR, RIGA 226067, USSR.
SOURCE: BIOTECHNOL BIOENG, (1980) 22 (4), 897-912.
CODEN: BIBIAU. ISSN: 0006-3592.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A scheme for lysine biosynthesis using variants of the B. flavum intermediary metabolite synthesis is discussed. The main precursor of lysine under consideration is oxalacetate, which can be synthesized through the TCA [tricarboxylic acid] or glyoxylate cycles or by carboxylation of PEP [phosphoenolpyruvate]. Material and energy balances for the main pathways of lysine biosynthesis from glucose and acetate were formulated. Energy consumption, in the form of ATP-PATP (number of mol ATP consumed/1 mol lysine synthesized), was calculated for the main pathways of lysine biosynthesis. Theoretical conversion yields Ypmax (g product/g substrate) were estimated. Experimental data were presented concerning the increase of Yp by metabolism regulation: by TCA- and glyoxylate cycle enzyme induction; by maintaining PEP carboxylase activity; by eliminating byproduct synthesis.

L4 ANSWER 24 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1980:205882 BIOSIS
DOCUMENT NUMBER: BA69:80878
TITLE: REGULATION OF PHOSPHOENOL **PYRUVATE CARBOXYLASE** EC-4.1.1.31 BY SYNERGISTIC ACTION OF ASPARTATE AND 2 OXO GLUTARATE.
AUTHOR(S): SHIIO I; UJIGAWA-TAKEDA K
CORPORATE SOURCE: CENT. RES. LAB., AJINOMOTO CO. INC., KAWASAKI, KANAGAWA, JPN.
SOURCE: AGRIC BIOL CHEM, (1979 (RECD 1980)) 43 (12), 2479-2486.
CODEN: ABCHA6. ISSN: 0002-1369.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB In accordance with the regulation by aspartate of phosphoenolpyruvate (PEP) carboxylase, glutamate formation in Brevibacterium flavum, a glutamate-producing bacterium, was inhibited by the addition of aspartate. An increase in the aspartate formation caused by a mutational decrease in citrate synthase specific activity was accompanied by a decrease in the total amount of glutamate and aspartate formed. A mutational decrease in glutamate dehydrogenase activity caused a decrease in the total amount without increasing the asparate formation but with accumulation of 2-oxoglutarate, suggesting that the feedback inhibition by the aspartate of PEP carboxylase was enhanced by 2-oxoglutarate. Partially purified PEP carboxylase from this organism was synergistically inhibited by aspartate and 2-oxoglutarate, citrate, cis-aconitase or isocitrate. Among them, the effects of tricarboxylic acids were attributed to their nonspecific chelating action with Mn2+, an activator of the enzyme. The synergistic action of 2-oxoglutarate was accompanied by a decrease in Hill coefficient for the aspartate of the enzyme.

L4 ANSWER 25 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1980:147872 BIOSIS
DOCUMENT NUMBER: BA69:22868
TITLE: ROLE OF BIOTIN DEPENDENT **PYRUVATE CARBOXYLASE** EC-6.4.1.1 IN L LYSINE PRODUCTION.
AUTHOR(S): OSAMU TOSAKA H M; TAKINAMI K
CORPORATE SOURCE: CENT. RES. LAB. AJINOMOTO CO., KAWASAKI, KANAGAWA, JPN.
SOURCE: AGRIC BIOL CHEM, (1979) 43 (7), 1513-1520.
CODEN: ABCHA6. ISSN: 0002-1369.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The promotive effect of biotin (200-500 .mu.g/l) on L-lysine formation was investigated in Brevibacterium lactofermentum. This effect was observed only when glucose or pyruvate was used as sole C source, and accompanied with the specific incorporation of 13CO2 into the .gamma.-CH2 group of L-lysine. B. lactofermentum AJ3445 (AECr) could grow on pyruvate medium supplemented with biotin at more than 200 .mu.g/l, while the same growth was observed with the addition of TCA [tricarboxylic acid cycle] members or glutamate to pyruvate medium. Phosphoenolypyruvate (PEP) carboxylase deficient mutants derived from AJ 3445 could not grow on glucose as sole C source, but could grow on glucose plus 200 .mu.g/l of biotin. AJ 3445 grown on lactate medium containing 500 .mu.g/l of biotin and KHCO3 contained the biotin-dependent **pyruvate carboxylase**.
This promotive effect of excess biotin on L-lysine formation may be brought about through the activation of **pyruvate carboxylase** by biotin.

L4 ANSWER 26 OF 28 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 80120091 MEDLINE
DOCUMENT NUMBER: 80120091 PubMed ID: 119147
TITLE: [Carboxylation enzymes of **coryneform** bacteria].
Fermenty karboksilirovaniia u korinepodobnykh bakterii.
AUTHOR: Baryshnikova L M; Loginova N V
SOURCE: MIKROBIOLOGIIA, (1979 Nov-Dec) 48 (6) 965-8.
Journal code: MZI; 0376652. ISSN: 0026-3656.
PUB. COUNTRY: USSR
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Russian
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198004
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19980206
Entered Medline: 19800417

AB The enzymes of carbon dioxide heterotrophic fixation were studied in six strains of **coryneform** bacteria belonging to the genera Arthrobacter, Brevibacterium, **Corynebacterium** and Nocardia. All of the strains were found to contain PEP (phosphoenolpyruvate) carboxylase (EC 4.1.1.31), NADP or NAD dependent malic enzymes (EC 1.1.1.38--40). **Pyruvate carboxylase** (EC 6.4.1.1) was found only in three strains of **coryneforms**: Brevibacterium ammoniagenes, **Corynebacterium** aquaticum and Nocardia erythropolis. PEP carboxykinase (EC 4.1.1.32) was detected in Brevibacterium ammoniagenes and Nocardia erythropolis. PEP carboxytransphosphorylase (EC 4.1.1.38) was found only in Brevibacterium ammoniagenes. These data suggest that carboxylation of C3-acids is one of the essential pathways in some **coryneforms** supplying the citric acid cycle with the products of glycolysis. The composition and the level of carboxylation enzymes reflect the ecological characteristics of the organisms rather than their taxonomical relations.

L4 ANSWER 27 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1980:142621 BIOSIS
DOCUMENT NUMBER: BA69:17617
TITLE: PYRUVATE AND PHOSPHOENOL PYRUVATE CARBOXYLASES OF METHYLOTROPHS.
AUTHOR(S): LOGINOVA N V; TROTSENKO YU A
SOURCE: MIKROBIOLOGIYA, (1979) 48 (2), 202-207.
CODEN: MIKBA5. ISSN: 0026-3656.
FILE SEGMENT: BA; OLD
LANGUAGE: Russian

AB The activity of pyruvate and phosphoenolpyruvate carboxylases was determined in cell extracts of obligate and facultative methylotrophs which metabolized monocarbon ruduced compounds via different pathways. Phosphoenolpyruvate carboxylase was the only enzyme responsible for the high level of CO2 fixation by methylotrophs with the serine pathway (Methylosinus trichosporium, Hyphomicrobium vulgare, Pseudomonas methylica). Methylotrophs with the hexulose phosphate pathway (Methylobacter chroococcum, Methylomonas methanica, P. oleovorans, Arthrobacter globiformis) and yeast (Candida methylica) assimilated less CO2 but contained more enzymes involved in arboxylation of phosphoenolpyruvate (phosphoenolpyruvate carboxylase, EC 4.1.1.31; phosphoenolpyruvate carboxykinase, EC 4.1.1.32) or pyruvate (**pyruvate carboxylase**, EC 6.4.1.1; malic-enzyme, EC 4.1.1.40). Phosphoenolpyruvate carboxytransphosphorylase (EC 4.1.1.38) was not found in any of the studied strains. The properties and the role of carboxylases in the metabolism of methylotrophs are discussed.

L4 ANSWER 28 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1979:163697 BIOSIS
DOCUMENT NUMBER: BA67:43697
TITLE: ENZYMES OF THE GLUTAMATE AND ASPARTATE SYNTHETIC PATHWAYS IN A GLUTAMATE PRODUCING BACTERIUM BREVIBACTERIUM-FLAVUM.
AUTHOR(S): SHIIO I; UJIGAWA K
CORPORATE SOURCE: CENT. RES. LAB., AJINOMOTO CO. INC., KAWASAKI, KANAGAWA 210, JPN.
SOURCE: J BIOCHEM (TOKYO), (1978) 84 (3), 647-658.
CODEN: JOBIAO. ISSN: 0021-924X.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Glutamate-auxotrophic mutants lacking phosphoenolpyruvate carboxylase (PC), citrate synthase (CS) or glutamate dehydrogenase (GD), an aspartate auxotroph lacking aspartate aminotransferase (TA) and a glutamate-aspartate double auxotroph lacking both aconitase (AH) and TA were obtained from B. flavum no. 2247, a glutamate-producing bacterium. Prototrophic revertants further derived from the CS- and GD-lacking auxotrophs concomitantly recovered the enzyme activities that their parents had lost. These results indicate involvement of the tricarboxylic

acid (TCA) cycle and GD in glutamate biosynthesis, that of PC in the biosynthesis of the TCA cycle intermediates and that of TA in aspartate biosynthesis. The CS-deficient mutants accumulated large amounts of acetate and small amounts of pyruvate, aspartate and alanine, while the GD-deficient strains accumulated large amounts of 2-oxoglutarate and small amounts of citrate. Synthesis of PC was repressed by either glutamate or aspartate and those of CS and GD were repressed by glutamate, but those of pyruvate dehydrogenase (PD), AH and isocitrate dehydrogenase were not affected significantly by glutamate; that of TA was also not affected by aspartate or by glutamate. The specific activities of PD and AH gave peaks during the cellular cultivation, related to the temporary accumulation of their substrates, pyruvate and citrate, respectively. These and previous results on the regulation of the enzymatic activities provide a definite regulatory mechanism for glutamate and aspartate syntheses.